

Q' -- Figure 7 depicts a multiple sequence alignment of DWF7/STE1 with known sequences for  $\Delta^7$  sterol C-5 desaturases. The GenBank accession numbers for the sequences are M62623 (*S. cerevisiae*) (SEQ ID NO:16), AB004539 (*Schizosaccharomyces pombe*) (SEQ ID NO:17), L40390 (*C. glabrata*) (SEQ ID NO:15), and AF105034 (DWF7/STE1, Arabidopsis) (SEQ ID NO:18). The conserved transmembrane domains and histidine clusters are boxed and labeled. The positions of the premature stop codons in *dwf7-1* and *dwf7-2* are indicated with filled circles. Histidine residues in each conserved histidine box are identified with filled triangles. A consensus sequence (SEQ ID NO:19) is shown in the bottom row of the alignment. Capital letters stand for residues conserved among all sequences, whereas lowercase letters mean  $\geq 50\%$  identical. Dashes indicate gaps introduced to maximize alignment. Multiple sequence alignment was performed using PILEUP in the Genetics Computer Group software (Madison, WI) with a gap creation penalty of 4 and a gap extension parameter of 1. The annotation of the aligned sequences was performed using the ALSCRIPT software (Barton (1993) Protein Eng. 6:37-40).--

Please replace the paragraph beginning at page 8, line 19, with the following rewritten paragraph:

Q<sup>2</sup> -- Figures 8A-8D (SEQ ID NO:20) depict the complete gene sequence of *dwf7*, denoted by a dark grey bar. The premature stop codons for *dwf7-1* and *dwf7-2* are shown with triangles at nucleotide positions 1552 and 322, respectively. The coding sequence and corresponding amino acid sequence are represented by a light grey bar. The mRNA sequence is represented by a black bar and is shown in three segments. The gene includes two introns (positions 369-735 and 1042-1395) and three exons.--

Please replace the paragraph beginning at page 8, line 25, with the following rewritten paragraph:

Q<sup>3</sup>

-- Figure 9 (SEQ ID NO:21) shows the amino acid sequence corresponding to the coding sequence designated in Figures 8A-8D. The polypeptide sequences corresponding to the *dwf7-2* and *dwf7-1* alleles occur at positions 1-60 (SEQ ID NO:24) and 1-230 (SEQ ID NO:25), respectively.--

Please replace the paragraph beginning at page 8, line 28, with the following rewritten paragraph:

Q<sup>4</sup>

-- Figures 10A-10F (SEQ ID NO:22) show the gene sequence of the *dwf7* homologue, *HDF7*. The coding sequence and corresponding amino acid sequence are shown in three segments (exons), occurring at positions 1506-1734, 2024-2329 and 2416-2720 of the figure. The 5' UTR is shown at positions 1-1505 and the 3' UTR occurs at positions 2721-2925.--

Please replace the paragraph beginning at page 9, line 3, with the following rewritten paragraph:

Q<sup>5</sup>

-- Figure 11 (SEQ ID NO:23) shows the amino acid sequence corresponding to the coding sequence designated in Figures 10A-10F. The polypeptide sequence corresponding to the *HDF7 dwf7* polypeptide occurs at positions 1-230 of the figure.--

Please replace the paragraph beginning at page 40, line 24, with the following rewritten paragraph:

Q<sup>6</sup>

-- PCR products amplified using primer sets derived from the cDNA sequence of *STEROL1* (*STE1*) were subjected to sequencing. To design sets of primers that do not fall in exon-intron junctions, we predicted possible splice sites by using the RNASPL program available at the internet site of Baylor College of Medicine (Houston, TX;

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<http://dot.imgen.bcm.tmc.edu:9331/seq-search/gene-search.html>). Primers were designed using the Primer Selection software of DNASTAR (DNASTAR Inc., Madison, WI). Oligonucleotide sequences 5' to 3' are CAGTGTGAGTAAT T TAGCAT TACTA (S5D\_FF) (SEQ ID NO:1), GGAAAGATCATC-AAACAT T TACATGT (S5D\_LR) (SEQ ID NO:2), GCGCAATCT TCT T TCGT T T (S5D\_1F) (SEQ ID NO:3), TGGACAACAACAACACAAGA (S5D\_1R) (SEQ ID NO:4), GATGCACAGAGAGCT- TCATGAC (S5D\_2F) (SEQ ID NO:5), CCGGCAAATGGAGAGAGTGTAT (S5D\_2R) (SEQ ID NO:6), CACCCATCATATCTACAACAA (S5D\_3F) (SEQ ID NO:7), and CATCT T T TGCCG-GCGAATCTAT (S5D\_4F) (SEQ ID NO:8) (underlines were added to distinguish forward or reverse primers from the gene acronym S5D). Primers were purchased from Genosys Biotechnologies, Inc. (The Woodlands, TX). For template DNA, genomic DNA was isolated from two or three leaves of *dwf7-1* and wild-type plants according to the method described by Krysan et al. (1996) Proc. Natl. Acad. Sci. USA 93:8145-8150. Amplification of the DNA fragment spanning the whole coding region was performed with the S5D\_4F and S5D\_1R primer set with Taq polymerase (Boehringer Mannheim).--

Please replace the paragraph beginning at page 42, line 3, with the following rewritten paragraph:

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-- Genomic DNA sequence flanking the cDNA was identified by sequencing the products obtained from thermal asymmetric interlaced PCR (TAIL PCR) (Liu et al. (1995) Plant J. 8:457-463). Two sets of primers were used to amplify the 5' and 3' flanking DNA. Oligonucleotide sequences 5' to 3' are GTAGAAGCACCAGAGGAAACCGGAGATGAAGT (D7-5-1; melting temperature of 69°C) (SEQ ID NO:9), AAGTATAGTAGGGT TCCGGCGAGG-TA (D7-5-2; melting temperature of 64°C) (SEQ ID NO:10), ATAGAT TCGCCG-GCAAAAGATGACTC

(D7-5-3; melting temperature of 63°C) (SEQ ID NO:11),  
TGC-AGGATACCATACGATACACCACACGACAT (D7-3-1; melting temperature of  
68°C) (SEQ ID NO:12), CATACGATACACCACACGACATACAAGCAT-AACTA  
(D7-3-2; melting temperature of 67°C) (SEQ ID NO:13), and ATATGGATG-GAT  
TGGATGT T TGGCTCTC (D7-3-3; melting temperature of 63°C) (SEQ ID NO:14).

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The melting temperature of each primer was calculated with the formula  $69.3 + 0.41$   
(%GC) - 650/L (Mazars et al. (1991) Nucleic Acids Res. 19:4783), where L is length of  
primer. Arbitrary degenerate primers AD1, AD2, and AD3 were synthesized according  
to the sequence described by Liu et al. (1995) Plant J. 8:457-463. TAIL PCR was  
performed according to the program originally described by Liu et al. 1995. TAIL  
PCR-amplified DNA was separated on 1% agarose gels and gel extracted for  
sequencing.--

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